REMARKS

1. Applicants hereby submit the following:

[ ] a paper copy of a "Sequence Listing", complying with §1.821(c), to be incorporated into the specification as directed above;

[XX] an amendment to the paper copy of the "Sequence Listing" submitted on November 13, 2001, the amendment being in the form of substitute sheets;

[XX] the Sequence Listing in computer readable form, complying with §1.821(e) and §1.824, including, if an amendment to the paper copy is submitted, all previously submitted data with the amendment incorporated therein;

[ ] pursuant to §1.821(e), reference is made to the computer readable form filed on , in USSN , which presents the identical Sequence information, the use of which is now requested, in lieu of submitting a new computer readable form; and/or

[ ] a substitute computer readable form to replace one found to be damaged or unreadable.

[XX] 2. The description has been amended to comply with §1.821(d).

3. The undersigned attorney or agent hereby states as follows:

(a) this submission is not believed to include new matter [§1.821(g)];

(b) the contents of the paper copy (as amended, if applicable) and the computer readable form of the Sequence Listing, are believed to be the same [§1.821(f) and §1.825(b)];

(c) if the paper copy has been amended, the amendment is believed to be supported by the specification and is not believed to include new matter [§1.825(a)]; and

(d) if the computer readable form submitted herewith is a substitute for a form found upon receipt by the PTO to be damaged or unreadable, that the substitute data is believed to be identical to that originally filed [§1.825(d)].

4. Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of

"Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free

sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The 21 paragraphs beginning at line 13 of page 7 and ending at line 3 of page 9 have been amended as follows:

Figure 1 shows the amino acid sequence (SEQ ID NO:15) (in one letter code) of human apolipoprotein A-I.

Figure 2A shows CLUSTAL W (1.74) multiple sequence alignment of apolipoprotein A-I using BLOSUM. The following sequences are aligned in the Figure:

HUMAN sp|P02647|APA1_HUMAN Apolipoprotein A-I precursor (Apo-AI) - Homo sapiens (Human) (SEQ ID NO:15).

Macaque sp|P15568|APA1_MACFA Apolipoprotein A-I precursor (Apo-AI) - Macaca fascicularis (Crab eating macaque) (SEQ ID NO:16).

Bovine sp|P15497|APA1_BOVIN Apolipoprotein A-I precursor (Apo-AI) - Bos taurus (Bovine) (SEQ ID NO:17).

Pig sp|P18648|APA1_PIG Apolipoprotein A-I precursor (Apo-AI) - Sus scrofa (Pig) (SEQ ID NO:18).

Dog sp|P02648|APA1_CANFA Apolipoprotein A-I precursor (Apo-AI) - Canis familiaris (Dog) (SEQ ID NO:19).

Rabbit sp|P09809|APA1_RABIT Apolipoprotein A-I precursor (Apo-AI) - Oryctolagus cuniculus (Rabbit) (SEQ ID NO:20).

Tree shreew sp|O18759|APA1_TUPGB Apolipoprotein A-I precursor (Apo-AI) - Tupaia glis belangeri (Common tree shrew) (SEQ ID NO:21).

Mouse sp|Q00623|APA1_MOUSE Apolipoprotein A-I precursor (Apo-AI) - Mus musculus (Mouse) (SEQ ID NO:22).

Rat sp|P04639|APA1_RAT Apolipoprotein A-I precursor (Apo-AI) - Rattus norvegicus (Rat) (SEQ ID NO:23).

Eur. Hedgehog tr|Q9TS49 APOLIPOPROTEIN A-I, APOA-I=CHOLESTEROL TRANSPORTER - Erinaceus europaeus (Western European hedgehog) (SEQ ID NO:24).

Chicken sp|P08250|APA1_CHICK Apolipoprotein A-I precursor (Apo-AI) - Gallus gallus (Chicken) (SEQ ID NO:25).

Jap. quail sp|P32918|APA1_COTJA Apolipoprotein A-I precursor (Apo-AI) - Coturnix coturnix japonica (Japanese quail) (SEQ ID NO:26).

Domestic duck sp|O42296|APA1_ANAPL Apolipoprotein A-I precursor (Apo-AI) - Anas platyrhynchos (Domestic duck) (SEQ ID NO:27).

Rainbow trout sp|O57523|AP11_ONCMY Apolipoprotein A-I-1 precursor (APOA-I-1) - Oncorhynchus mykiss (Rainbow trout) (Salmo gairdneri) (SEQ ID NO:28).

Brown trout sp|Q91488|APA1_SALTR Apolipoprotein A-I precursor (Apo-AI) - Salmo trutta (Brown trout) (SEQ ID NO:29).

Atl. salmon sp|P27007|APA1_SALSA Apolipoprotein A-I precursor (Apo-AI) - Salmo salar (Atlantic salmon) (SEQ ID NO:30).

Zebrafish sp|O42363|APA1_BRARE Apolipoprotein A-I precursor (Apo-AI) - Brachydanio rerio (Zebrafish) (Zebra danio) (SEQ ID NO:31).

Sea bream sp|O42175|APA1_SPAAU Apolipoprotein A-I precursor (Apo-AI) - Sparus aurata (Gilthead sea bream) (SEQ ID NO:32).

Figure 2B shows aligned amino acid sequences (in one letter code) for human (SEQ ID NO:33), macaque (SEQ ID NO:34), mouse (SEQ ID NO:35), baboon (SEQ ID NO:36), pig (SEQ ID NO:37), and rat (SEQ ID NO:38) apolipoprotein A-IV.

The 7 paragraphs beginning at line 11 of page 9 and ending at line 15 of page 10 have been amended as follows:

Figure 4 shows an alignment of the amino acid sequences of the trimerising structural element of the tetranectin protein family. Amino acid sequences (one letter code) corresponding to residue V17 to K52 comprising exon 2 and the first three residues of exon 3 of human tetranectin (SEQ ID NO:39); murine tetranectin (SEQ ID NO:40) (Sørensen et al., Gene, 152: 243 -245, 1995); tetranectin homologous protein isolated from reefshark cartilage (SEQ ID NO:42)

(Neame and Boynton, 1992,1996); and tetranectin homologous protein isolated from bovine cartilage (SEQ ID NO:41) (Neame and Boynton, database accession number PATCHX:u22298). Residues at a and d positions in the heptad repeats are listed in boldface. The listed consensus sequence of the tetranectin protein family trimerising structural element comprise the residues present at a and d positions in the heptad repeats shown in the figure in addition to the other conserved residues of the region. "hy" denotes an aliphatic hydrophobic residue.

Figure 5 shows the pT7 H6UbiFx Apo A-I plasmid (SEQ ID NO:43) and its corresponding amino acid sequences (SEQ ID NO:44). The expressed and processed polypeptide consists of amino acids no 25-267 from human Apo A-I (SEQ ID NO 1) and gly-gly linked N-terminally thereto.

Figure 6 shows the pT7 H6UbiFx Cys-Apo A-I plasmid (SEQ ID NO:45) and its corresponding amino acid sequences ~~for~~ (SEQ ID NO:46). The expressed and processed polypeptide consists of a N-terminal cystein residue and the amino acids no 25-267 from human Apo A-I (SEQ ID NO 2) and gly-gly linked N-terminally thereto.

Figure 7 shows the pT7H6 Trip-A-Apo A-I - Amp^R plasmid (SEQ ID NO:47) and its corresponding amino acid sequence (SEQ ID NO:48). The expressed and processed

polypeptide (SEQ ID NO 3) consists of the tetranectin trimerising structural element (TTSE), a linking sequence, and amino acids no 25-267 from human Apo A-I.

Figure 8 shows the pT7H6 Trip-A-Apo A-I-del 43 - Amp^R plasmid (SEQ ID NO:49) and its corresponding amino acid sequence (SEQ ID NO:50). The expressed and processed polypeptide (SEQ ID NO 4) consists of the TTSE, a linking sequence, and amino acids no 68-267 from human Apo A-I.

Figure 9 shows the pT7H6FXCysApoAI plasmid (SEQ ID NO:51) and its corresponding amino acid sequence (SEQ ID NO:52). The expressed and processed polypeptide consists of a N-terminal cystein residue and the amino acids no 25-267 from human Apo A-I (SEQ ID NO 2) and gly-gly linked N-terminally thereto.

Figure 10 A to G shows illustrative examples of plasmids (SEQ ID NOs:53, 55, 57, 59, 61, 63 and 65, respectively) and corresponding amino acid sequences (SEQ ID NOs:54, 56, 58, 60, 62, 64 and 66, respectively) for apolipoprotein constructs according to the present invention.

The paragraph beginning at line 23 of page 11 has been amended as follows:

Fig 10 H: pT7H6Fx-Hp(alpha)-ApoAI. The plasmid (SEQ ID NO:67) codes for the fusion protein (SEQ ID NO:68) between

Hp(alpha) and ApoAi. The mature protein product is called Hp(alpha)-ApoAI (SEQ ID NO 14).

The four paragraphs beginning at line 17 of page 27 and ending at line 19 of page 28 have been amended as follows:

Tetranectin based linker:

The linker may include the tetranectin residues 53-56, which in tetranectin forms a β-strand, and the residues 57-59 which forms a turn in tetranectin (Nielsen BB, Kastrup JS, Rasmussen H, Holtet TL, Graversen JH, Etzerodt M, Thøgersen HC, Larsen IK, FEBS-Letter 412, 388-396, 1997). The sequence of the segment is GTKVHMK (SEQ ID NO:69). This linker has the advantage that it in native tetranectin is bridging the trimerisation domain with the CRD-domain, and hence is imagined to be well suited for connecting the trimerisation domain to another domain in general. Furthermore the resulting construct is not expected to be more immunogenic than the construct without a linker. The tetranectin based linker is highly preferred when the component X comprises the TTSE.

Fibronectin based linker:

The linker may be chosen as a sub-sequence from the connecting strand 3 from human fibronectin, this corresponds to amino acid residues 1992-2102 (SWISS-PROT numbering, entry

P02751). Preferably the subsequence: PGTSGQQPSVGQQ (SEQ ID NO:70) covering amino acid residues number 2037-2049 is used, and within that subsequence the segment GTSGQ (residues 2-6 of SEQ ID NO:70) corresponding to amino acid residues 2038-2042 is more preferable. This construct has the advantage that it is know not to be highly prone to proteolytic cleavage and is not expected to be highly immunogenic bearing in mind that fibronectin is present at high concentrations in plasma.

Human IgG_3 upper hinge based linker

The 10 amino acid residue sequence derived from the upper hinge region of murine IgG_3, PKPSTPPGSS (SEQ ID NO:71), has been used for the production of antibodies dimerised trough a coiled coil (Pack P. and Plückthun, A. Biochemistry **31**, pp 1579-1584 (1992)) and may be useful as a spacer peptide according to the present invention. Even more preferable may be a corresponding sequence from the upper hinge region of human IgG_3. Sequences from human IgG_3 are not expected to be immunogenic in human beings.

Flexible linkers

Possible examples of flexible linker/spacer sequences include SGGTSGSTSGTGST (SEQ ID NO:72), AGSSTGSSTGPGSTT (SEQ ID NO:73) or GGSGGAP (SEQ ID NO:74). These sequences have been used for the linking of designed

coiled coils to other protein domains (Müller, K. M., Arndt, K. M. and Alber, T., Meth. Enzymology, **328**, pp 261-281 (2000).

The paragraph beginning at line 14 of page 41 has been amended as follows:

The cDNA encoding Apo A-I was amplified from a human liver cDNA library (Clontech) using standard PCR techniques. For the construction of Ubi-A-I the primers used were: 5′-CAC GGA TCC ATC GAG GGT AGG GGT GGA GAT GAA CCC CCC CAG AGC-3′ (SEQ ID NO:75) and 5′- TCC AAG CTT ATT ACT GGG TGT TGA GCT TCT TAG TG-3′ (SEQ ID NO:76). The product was cloned into the vector pT7H6Ubi, described in (Ellgaard L. et al Eur. J. Biochem. 1997;244(2):544-51) using the Bam HI and Hind III cloning sites. For the construction of Trip-A-A-I the primers used were 5′-AAG GGA TCC GAT GAA CCC CCC CAG AGC CCC-3′ (SEQ ID NO:77) and 5′-TCC AAG CTT ATT ACT GGG TGT TGA GCT TCT TAG TG-3′ (SEQ ID NO:78). The PCR product was cloned into the pT7H6tripa vector described in WO 98/56906 using the Bam HI and Hind III cloning sites. For the construction of Trip-A-I-del43 the primers used were 5′-AGG GGA TCC CTA AAG CTC CTT GAC AAC TGG G-3′ (SEQ ID NO:79) and 5′- TCC AAG CTT ATT ACT GGG TGT TGA GCT TCT TAG TG -3′ (SEQ ID NO:80). The PCR product was cloned into the pT7H6tripa vector described in WO 98/56906 using the Bam HI and Hind III cloning sites. For the

construction of Ubi-Cys-A-I the primers used were: 5´-GGT GGA TCC ATC GAG GGT AGG GGT GGA TGT GAT GAA CCC CCC C -3´ (SEQ ID NO:81) and 5´- TCC AAG CTT ATT ACT GGG TGT TGA GCT TCT TAG TG -3´ (SEQ ID NO:82). The product was cloned inti the vector pT7H6Ubi, described in (Ellgaard L. et al Eur. J. Biochem. 1997;244(2):544-51) using the Bam HI and Hind III cloning sites. The plasmids generated are shown on figure 4, 5, 6, and 7.

The paragraphs beginning at line 34 of page 46 and ending at line 17 of page 47 have been amended as follows:

pT7H6FX-Trip-A-FN(-2)-AI:

5´-CGC GGATCC TCG GGT CAG GAT GAA CCC CCC CAG AGC CCC -3' (SEQ ID NO:83)

Unfortunately all the isolated clones had the above highlighted **G** mutated to a T, indicating a faulty sequence of the primer.

pT7H6FX-Trip-A-TN-AI-Bam-S

5'- cgc gga tcc aag gtg cac atg aag gat gaa ccc ccc cag agc ccc-3' (SEQ ID NO:84)

The mutations mentioned was corrected by site directed mutagenesis using the QuickChange kit from Stratagene and the following sets of primers:

pT7H6FX-Trip-FN-AI:

5′-acg gtc tcc ctg aag gga acc tcg ggt cag gat g-3′ (SEQ ID NO:85)

5′-cat cct gac ccg agg ttc cct tca ggg aga ccg t-3′

pT7H6FX-Trip-A-TN-AI

5′-acg gtc tcc ctg aag gga acc aag gtg cac atg aag g-3′ (SEQ ID NO:86)

5′-cct tca tgt gca cct tgg ttc cct tca ggg aga ccg t-3′

The paragraphs beginning at line 26 of page 47 and ending at line 33 of page 47 have been amended as follows:

For the mutation of lysine 9 from Trip-A:

5′-cca acc cag aag ccc aag gcg aat gta aat gcc-3′ (SEQ ID NO:87)

5′-gtg ttc aca aca tct gcc ttg gca ttt aca atc-3′ (SEQ ID NO:88)

For the mutation of lysine 15 from Trip-A:

5′-ggc att tac aat cgc ctt ggg ctt ctg ggt tgg-3′ (SEQ ID NO:89)

5′-cca acc cag aag ccc aag gcg att gta aat gcc-3′

The paragraph beginning at line 16 of page 48 has been amended as follows:

From a cDNA library (Clontech fetal liver) the Hp-alpha sequence was PCR amplified using the following set of primers:

Non-sense primer: 5'-cac aag ctt tcc gct aga tct ctg cac tgg gtt agc cgg att ctt ggg -3' (SEQ ID NO:90)

Sense Primer: 5'-ggt gga tcc atc gag ggt agg ggt gtg gac tca ggc aat gat gtc acg g-3' -3' (SEQ ID NO:91)

In the claims:

Claims 5 and 42 have been amended as follows:

5 (Amended). The composition according to claim 2, wherein the spacer peptide comprises the amino acid sequence GTKVHMK (SEQ ID NO:69) from tetranectin, amino acid sequence PGTSGQQPSVGQQ (SEQ ID NO:70) and GTSGQ (residues 2-6 of SEQ ID NO:70) from the connecting strand 3 from human fibronectin, PKPSTPPGSS (SEQ ID NO:71) from the upper hinge region of murine IgG_3, SGGTSGSTSGTGST (SEQ ID O:72), AGSSTGSSTGPGSTT (SEQ ID NO:73) or GGSGGAP (SEQ ID NO:74).

42 (Amended). The construct according to claim 39, wherein the spacer peptide comprises the amino acid sequence GTKVHMK (SEQ ID NO:69) from tetranectin, amino acid sequence PGTSGQQPSVGQQ (SEQ ID NO:70) and GTSGQ (residues 2-6 of SEQ ID NO:70) from the connecting strand 3 from human fibronectin, PKPSTPPGSS (SEQ ID NO:71) from the upper hinge region of

murine IgG_3, SGGTSGSTSGTGST (SEQ ID NO:72), AGSSTGSSTGPGSTT (SEQ ID NO:73) or GGSGGAP (SEQ ID NO:74).